

REMARKS

Claims 3-7, 12, 13 and 22-39 are pending. This Amendment B amends Claims 3, 12 and 31. Reconsideration of the pending claims is respectfully requested in view of the claim amendments above and the discussion below.

A. 35 USC § 103(a) Rejection

The Office has asserted the following obviousness-based reasons for rejecting the claims:

(a) Claims 3, 6, 7, 26, 29, 30, and 34-36 were rejected under 35 USC § 103(a) as unpatentable over Thomas et al. (J. Allergy Clin. Immunol., vol. 99, no. 1, p. S187, January 24, 1997) (the "Thomas reference") in view of Francis and Clarke (Methods in Enzymology, vol. 178, pp. 659-676, 1989) (the "Francis reference");

(b) Claims 3, 6, 7, 22, 23, 26-31, and 33-36 were rejected under 35 USC § 103(a) as unpatentable over Rittershaus and Thomas (WO 96/34888, November 7, 1996) (the "Rittershaus reference") in view of Donnelly et al. (J. Immunological Methods, vol. 176, pp. 145-152, 1994) (the "Donnelly reference") and further in view of the Francis reference:

(c) Claims 4, 5, and 37-39 were rejected under 35 USC § 103(a) as unpatentable over the Thomas reference in view of the Francis reference and further in view of the Donnelly reference; and

(d) Claims 12, 13, and 32 were rejected under 35 USC § 103(a) as unpatentable over the Thomas reference in view of Brown et al. (Vaccine, vol. 9, pp. 595-601, 1991).

The Rittershaus reference was the basis for rejection in the parent application, U.S. Patent Application Serial No. 08/934,367 filed September 19, 1997, as well as in grandparent applications U.S. Patent Application Serial No. 08/785,997 filed January 21, 1997, and U.S. Patent Application Serial No. 08/788, 882 filed January 21, 1997. Declarations pursuant to 37 C.F.R. §1.131 were filed in each of those applications to overcome this reference. True and correct copies of the Declarations filed in the parent and grandparent applications are attached to this Amendment B as Exhibits A

and B and are incorporated by reference in this Amendment B as if fully set forth at length.

Paragraph 7 of each Declaration asserts that the work described in the Declaration and forming the basis for the pending application was completed in this country prior to November 7, 1996. The Thomas reference cited by the Office has a nominal publication date of January 24, 1997. The Rittershaus reference cited by the Office has a PCT publication date of November 7, 1997 (not November 2, 1997 as noted in the May 22, 2000 Office action). Accordingly, the Declarations remove the Thomas reference and the Rittershaus reference as prior art references against the pending application and the rejections have been overcome.

B. 35 USC § 112, Second Paragraph Rejection

The Office rejected Claims 3-7, 12, 13, and 22-39 under 35 USC § 112, second paragraph, for failing to particularly point out and distinctly claim the subject matter that the Applicants regard as the invention. Reconsideration of these rejections is respectfully requested.

First, the terms "cholesteryl ester transfer protein" and its abbreviation "CETP" as used in the claims are not indefinite. A cholesteryl ester transfer protein ("CETP") is clearly defined in the application as an acid plasma glycoprotein that mediates the transfer of cholesteryl esters from HDL particles to LDL and VLDL particles and also mediates the transfer of triglycerides from LDL and VLDL to HDL particles. Application, page 1, lines 25-26 ; and page 1, line 34 through page 2, line 1. The CETP amino acid residue and nucleotide sequences of mammalian species have been characterized and are known in the art. Application, page 2, line 30 through page 3, line 10. In fact, the specific CETP sequences for human, rabbit and cynomolgus monkey species are discussed in the application and are disclosed in the literature. *Id.* While the term "cholesteryl ester transfer protein" may be broad, there is nothing indefinite about that term. One of ordinary skill in the art would readily understand its meaning as used in the claims.

Second, the term "antigenic carrier" used in Claim 12 does have antecedent basis. Claim 3 (from which Claim 12 depends) was previously amended by the

Preliminary Amendment received by the Office on March 7, 2000, and presently provides the necessary antecedent basis.

Third, Claim 12 has been amended by the present Amendment B to provide antecedent basis for the term "encoded fusion protein" used in Claim 13.

Fourth, Claim 3 has been amended to correct an editorial error and clarify that the process of that claim increases (not decreases) blood HDL levels.

Fourth, the Office has rejected Claim 28 on the basis that it improperly depends from Claim 35. Applicants respectfully request the Office to resequence and renumber Claim 28 in the appropriate manner once the claims are otherwise in condition for allowance.

Each of the above-discussed amendments was made solely to clarify the ambiguities in the claim language noted by the Office.

C. Obviousness-Type Double-Patenting Rejection

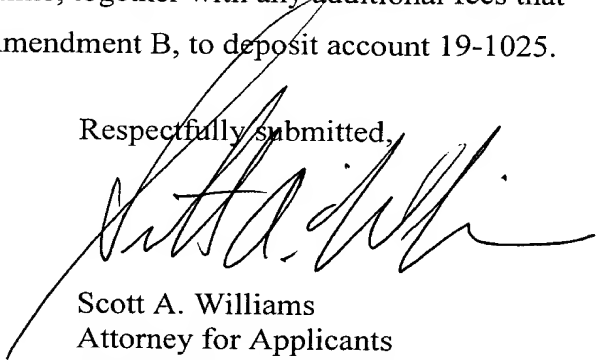
The Office has provisionally rejected Claims 3-7, 12, 13, and 22-39 under the doctrine of obviousness-type double patenting as being unpatentable over claims 3-7, 8-1, and 15-31 of co-pending Application Serial No. 08/934,367. Once the Office has indicated that the claims are allowable, Applicants will file the necessary terminal disclaimer (if necessary and appropriate at that time) to overcome the obviousness-type double patenting rejection.

In view of the foregoing amendments, applicants respectfully submit that the claims are in condition for allowance. Favorable consideration and early allowance of pending Claims 3-7, 12, 13, and 22-39 are therefore requested. Applicants further request a three month extension of time under 37 CFR 1.136 through November 22, 2000, for filing the present response to the May 22, 2000 Office action. The Commissioner is hereby authorized to charge the \$890.00 fee under 37 CFR 1.17(a)

Serial No. 09/386,591

required for the three month extension of time, together with any additional fees that may be required in connection with this Amendment B, to deposit account 19-1025.

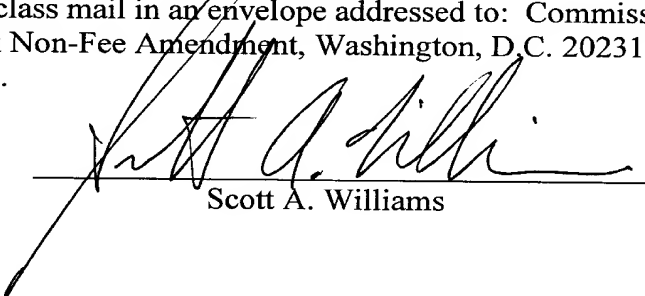
Respectfully submitted,


Scott A. Williams
Attorney for Applicants
Registration No. 39,876
314-694-4474

Pharmacia Corporation
Corporate Patent Department
P. O. Box 5110
Chicago, IL 60680-9889

CERTIFICATE OF MAILING

I certify that this AMENDMENT B in the application of Needleman et al., Serial No. 09/386,591, filed August 31, 1999, is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Box Non-Fee Amendment, Washington, D.C. 20231, on this 22nd day of November, 2000.



Scott A. Williams



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Needleman et al.)	Attorney Docket
)	6221/76429
Serial No.: 09/386,591)	Mon-103.0 CON I
)	
Filed: 31 Aug, 1999)	Art Group: 1642
)	
For: AN IMMUNOLOGICAL PROCESS AND)	
CONSTRUCTS FOR INCREASING)	
HDL CHOLESTEROL)	
CONCENTRATION BY DNA)	
VACCINATION)	

Examiner: J. Anders

November 22, 2000

EXHIBIT A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



Applicant: Needleman et al.)
Serial No.: 08/788,882) Attorney Docket
Filed: January 21, 1997) 6221/69242
For: AN IMMUNOLOGICAL PROCESS AND) MON-102.0
CONSTRUCTS FOR INCREASING) Art Group:
THE HDL CHOLESTEROL CONCENTRATION) 1642
Examiner: T. Scheiner)

DECLARATION OF PHILIP NEEDLEMAN AND
KEVIN GLENN PURSUANT TO 37 C.F.R. §1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PHILIP NEEDLEMAN, Ph.D. and KEVIN GLENN, Ph.D.

Declare:

1. That they are the Philip Needleman and Kevin Glenn that are the named inventors of the subject patent application;
2. That they are employed by G.D. Searle, Co., (Searle) a wholly owned subsidiary of the Monsanto Company (Monsanto), the assignee of the above-identified patent application;
3. That Dr. Needleman holds the positions of Senior Vice President of Research and Development at Searle and Chief

Scientist at Monsanto, whereas Dr. Glenn holds the titles of Fellow and Group Leader of Atherosclerosis Research at Searle;

4. That Elaine Krul, Ph.D., who is presently employed by the Nutrition and Consumer Sector, a division of the Monsanto Company, was formerly employed by Searle between February of 1994 and February of 1998 doing laboratory research related to atherosclerosis;

5. That during her employment by Searle, she carried out her research under the direction and control of one or both of Drs. Needleman and Glenn;

6. That the work described in this Declaration was carried out by Dr. Krul or a person under her direction and control, and was carried out in the State of Missouri, the United States of America;

7. That the work described in this Declaration was completed on a date prior to November 7, 1996, the date of publication of WO 996/34888;

8. That the documents attached to this Declaration are true copies of one or more of Dr. Krul's laboratory notebooks and an invoice, except that the dates and notebook numbers have been obscured for the purposes of this Declaration;

9. That collective Exhibit 1 contains pages 158, 159 and 160 from one of her notebooks (notebook A) that contains the amino acid residue sequence of cholesteryl ester transfer protein (CETP), using single letter code, in which peptide sequences corresponding to RABCTP-2, RABCTP-3, RABCTP-4, RABCTP-5, RABCTP-6, and RABCTP-7 are underlined and identified as such;

10. That those peptides of Paragraph 9 correspond to SEQ ID Nos: 2, 3, 4, 5, 6, and 7, respectively of the above-identified application;

11. That enclosed Exhibit 2 is a copy of page 161 of that same notebook that reiterates the names of those polypeptides and provides a schematic of a branched oligolysine antigenic carrier molecule referred to a Multiple Antigenic Peptide Backbone that when conjugated to a polypeptide is also referred to as MAP in her notebooks, and is noted at page 18, lines 11-19 of the above-identified application;

12. That enclosed Exhibit 3 is a copy of an invoice from Genosys Biotechnologies, Inc., of The Woodlands, Texas (Genosys) directed to Dr. Krul for conjugates of MAP-covalently-bonded polypeptides RABCTP-2, -3, -4, -5, -6 and -7;

13. That twelve rabbits were immunized on two occasions, two each, with a MAP-linked polypeptide conjugate received from Genosys;

14. That those two immunizations were recorded on pages 165, 166 and 167 of her above notebook, copies of which are enclosed as collective Exhibit 4;

15. That the samples of the immunized rabbits' blood were taken thereafter and were assayed for total cholesterol (TC) and high density lipoprotein (HDL-C);

16. That enclosed Exhibit 5 is a copy of page 071 of a second of her notebooks (notebook B) that contains graphs of the total cholesterol (TC) and high density lipoprotein (HDL-C) for each of the rabbits used in the study from prior to the immunizations (day zero) through at least 75 days thereafter, and in which the boxes within each graph identify each peptide with a shortened designation (CTP-number rather than RABCTP-number) because of space considerations;

17. That those data indicate a general lowering of both total cholesterol and high density lipoprotein for each of the peptides used;

18. That total cholesterol was determined in a standard laboratory assay commercially available from Wako Pure

Chemical Industries, Inc., adapted to a 96-well microtiter plate format;

19. That high density lipoprotein was determined in a standard laboratory assay using a commercially available kit from Sigma Chemicals, Inc., of St. Louis, MO;

20. That enclosed Exhibit 6 is a copy of page 072 of her notebook B showing data for rabbits immunized with conjugates of free polypeptides RABCTP-2, -3, -4, -6 and -7 (again referred to as CTP-2, CTP-3 etc.), also obtained from Genosys, that were individually covalently bonded to tuberculin purified protein derivative (PPD) to form conjugates, and prepared as discussed in Example 1B of the above-identified application, beginning at page 37;

21. That the results shown in Exhibit 6 were obtained following two immunizations with the conjugates using the assay procedures discussed in relation to Exhibit 5;

22. That all statements made herein of his (their) own knowledge are true and all statements made on information and belief are believed to be true; and further, these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the

United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Enclosures
Exhibits 1-6

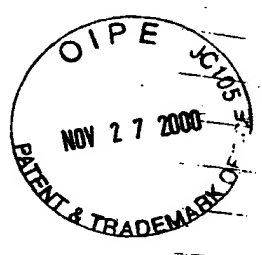
2/1/99
Date


Philip Needleman

6 January, 1999
Date


Kevin Glenn

RABBIT CETP SEQUENCE - RABCTP-5 / RABCTP-6 / RABCTP-7



RABCTP-7

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAQFQAGYPDVSGERAVM

10

20

30

40

50

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVFKGTLNYS

60

70

80

90

100

YTSAWGLQINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL

110

120

130

140

150

RABCTP-6

LLHLQGEREPGWLKQLETFNFISFTLKLILKGQVCNEINTISNIMADFEVQT

160

170

180

190

200

RAASILSDGDIGVDISVTGAPVITATYLESHHKHGFTHKNVSEAFPLRAF

210

220

230

240

250

PPGLLGDSRMLYFWFSDQVLNSLARAAFQEGRLVLSLTGDEFKKVLETQG

260

270

280

290

300

EDTNQEIFOELSRGLPTGQAQVAVHCLKVPKISCONRGVVVSSSVAVTFR

310

320

330

340

350

RABCTP-5

FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQVCPASGRAGSS

360

370

380

390

400

ANLSVALRTEAKAVSNLTESRSESLSLRSLIATVGIPEVMSRLEVAFT

410

420

430

440

450

ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHLVDFLQSL

460

470

480

490

SEARLE

Rabbit Anti-CETP Project
Choice of Peptides for Immunization

159

RABBIT CETP SEQUENCE - RABCTP-3 / RABCTP-4

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQAGYPDVSGERAVM
 10 20 30 40 50
 LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVFKGTLNYS
 60 70 80 90 100
 YTSAWGLGINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL
 110 120 130 140 150
 LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFVQT
 160 170 180 190 200
 RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF
 210 220 230 240 250
 PPGLLGDSRMLYEFWFSQVLNSLARAAFQEGRLVLSLTGDEFKKVLETQG
 260 270 280 290 300
RABCTP-3
 FDTNQEIFQELSRGLPTGQAQVAVHCLKVPKISQNRGVVVSSSVAVTER
 310 320 330 340 350
FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCVPASGRAGSS
 360 370 380 390 400
 ANLSVALRTEAKAVSNLTESRSESLSLRS LIATVG IPEVMSRLEVAFT
 410 420 430 440 450
 ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHLLVDFELQSL
 460 470 480 490
RABCTP-4

RABBIT CETP SEQUENCE - RABCTP-2

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAQFQAGYPDVSGERAVM

10 20 30 40 50

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVFKGTLNYS

60 70 80 90 100

YTSAWGLGINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL

110 120 130 140 150

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFVQT

160 170 180 190 200

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF

210 220 230 240 250

PPGLLGDSRMLYFWFSDQVLNSLARAQFQEGRLVLSLTGDEFKKVLETQG

260 270 280 290 300

FDTNQEIFQELSRGLPTGQAQVAHCLKVPKISCQNRGVVSSSVAVTFR

310 320 330 340 350

FPRPDGREAVAYRFEEDIITTVQASYSQKKLELHLLDFQCVPASGRAGSS

360 370 380 390 400

ANLSVALRTEAKAVSNLTESRSESLQSSLRSLIATVGIPEVMSRLEVAFT

410 420 430 440 450

ALMNSKGLDLFEIINPEIITLDGCLLQMDGFGPKHLLVDFLQSL

460 470 480 490

ture

Aine Kuel

Read and Understood By

S. Ragg

D--

Summary of Peptide Sequences Submitted to Genosys
to couple on MAP:

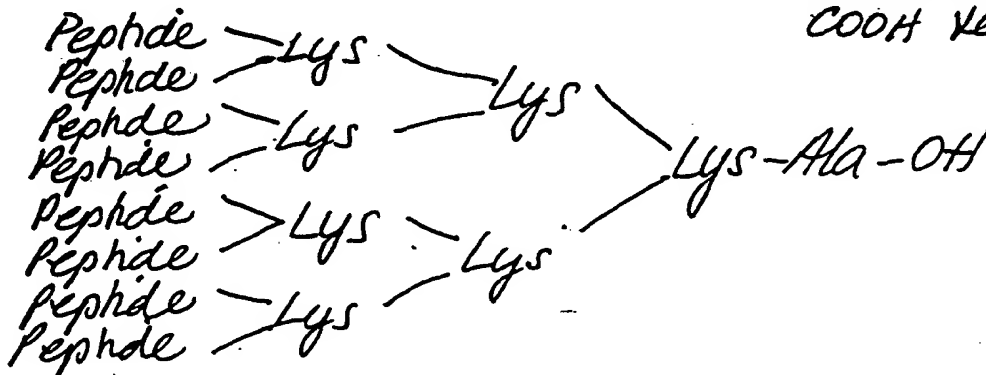
RABCTP-2	306-325	(#001)	Order # 054215 P.O.# 8002455
RABCTP-3	345-364	(#002)	40µM of each Made - Immuno
RABCTP-4	475-496	(#003)	Grade (>60% pure)
RABCTP-5	370-389	(#004)	Made on MAPS Resin: See reference: Butz, S. et al. (1994) Peptide Research 7:20-23.
RABCTP-6	150-169	(#005)	
RABCTP-7	42-61	(#006)	

Decided to try coupling to Multiple Antigenic Peptide
Backbone as a way to avoid "carrier" protein
which will generate a lot of antibodies of and
in itself.
One of the original references to this method is:

Posnett, DN, ~~McGrath~~ McGrath H, & Tam JP (1988)
J. Biol. Chem. 263: 1719-1725.

Structure of MAP:

NOTE: Peptides are
linked via their
COOH termini!!



Dr's Signature

Blaine Kruel

Read and Understood By

Sam

GENOSYS 1-800-234-3600
 Genosys Biotechnologies, Inc.
 1442 Lake Front Circle, Suite 185
 The Woodlands, TX 77380-3600
 (713) 363-3693
 FAX (713) 363-2212

Stacey ext #127
 Hogue

[615-343-1465]
 James Tam

Order No.

7m 1g
 0.37µm/mg norm

Dr. Stanfield

Page 1
 713-363-3693 ext 13

ORDER

54215

Bill To
 MONSANTO COMPANY
 ACCOUNTS PAYABLE. N2F
 800 NORTH LINDBERGH BLVD.

ST. LOUIS

MO 63167

Ship To
 DR. ELAINE KRULL
 MONSANTO COMPANY
 60-000-760.92
 T213W/T2M
 800 N. LINDBERGH BLVD.
 ST. LOUIS

MO 63167

CONTACT-----
 BARB GRIFFARD
 314-694-1000 X 6825

-----CONTACT-----
 314-694-4218

Customer: 100 6316701 KRUI
 Salesman: 8
 Terms: NET 30
 SHIP VIA: Airborne (Prepaid)
 J Number: B002455

ORDERED:

SHIPPED:
 INVOICED:

ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC	NET PRICE	EXTENSION
.000	1.00	0.00 PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUO GRADE RABCTP-2 EIF QEL SRG LPT GQA QVA VH RESIDUES IMMUO GRADE MAPS RESIN	20.00	35.00			~87 mg
			MW 2181					
			st P157A-022					
			hde # 54215-001					
.000	1.00	0.00 PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUO GRADE RABCTP-3 YAV TFR FPR PDG REA VAY RF RESIDUES IMMUO GRADE MAPS RESIN	20.00	35.00			~94 mg
			MW 2355					
			st P157A-024					
			phde # 54215-002					
.000	1.00	0.00 PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUO GRADE RABCTP-4 LLL QND FGF PKH LLV DFL QSL S RESIDUES IMMUO GRADE MAPS RESIN PEP SYN	22.00	35.00			~102 mg
			MW 2562					
			st # P157A-026					
			phde # 54215-003					
.000	1.00	0.00 PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUO GRADE RABCTP-5 TTV QAS YSQ XXL FLH LLD FQ RESIDUES IMMUO GRADE MAPS RESIN	20.00	35.00			~95 mg
			MW 2368					
			st P157A-028					
			phde # 54215-004					

ACKING LIST

GENOSYS

Genosys Biotechnologies, Inc.
1442 Lake Front Circle, Suite 185
The Woodlands, TX 77380-3600
(713) 363-3693
FAX (713) 363-2212

Order No.

Page 2

ORDER

54215

Bill T
MONSANTO COMPANY
ACCOUNTS PAYABLE, N2F
300 NORTH LINDBERGH BLVD.

ST. LOUIS MO 63167

Ship To
DR. ELAINE KRULL
MONSANTO COMPANY
60-000-760.92
T213W/T2M
800 N. LINDBERGH BLVD.
ST. LOUIS MO 63167

CONTACT
BARB GRIFFARD
314-694-1000 X 6825

CONTACT
314-694-4218

Customer: 100 6316701 KRUI
Sal sman: 8
Terms: NET 30
SHIP VIA: Airborne (Prepaid)
Number: B002455

ORDERED:

SHIPPED:

INVOICED:

ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC	NET PRICE	EXTENSION
.000	1.00	0.00 PEP1G/40	CUSTOM PEPTIDE 40 uM IMMUNO GRADE RABCTP-6 LLL HLQ GER EPG MLK QLF TH RESIDUES IMMUNO GRADE MAPS RESIN	20.00	35.00		950.00	
.000	1.00	0.00 PEP1G/40	CUSTOM PEPTIDE 40 uM IMMUNO GRADE RABCTP-7 DVS GER AVH LLG RVK YGL HH RESIDUES IMMUNO GRADE MAPS RESIN	20.00	35.00		950.00	

MENTS:
cleaned by 95% TFA, ether extracted 3x, suspended in
MoTFA/H₂O - 1-2% acetic acid suspended
+ lyophilized
4.624.00
TOTAL

ACKING LIST

SEARLE

Rabbit anti-CETP Project
Immunization Procedure

165

Immunization of Rabbits

Subcutaneously administered (0.05-0.1 ml/site)
along rabbit's back - done by Margi Baldwin.

Rabbits in Room 105 (Tattooed)

#1, #2	RABCTP-2	(3.3mg total ÷ 2 rabbits)	
#3, #4	RABCTP-3	(3.4mg ")
#5, #6	RABCTP-4	(3.2mg ")
#7, #8	RABCTP-5	(3.2mg ")
#9, #10	RABCTP-6	(3.3mg ")
#11, #12	RABCTP-7	(3.5mg ")

INVESTIGATOR: _____ DATE: _____
 room #: _____ phone # _____ Mail Zone: Tam
 Technician: _____

me
l
ked

1
n in
wbs
200C.

Animal ID #	Bleed	Serum/Plasma	Other	Initials
1	10	---	---	H
2	10	---	---	H
3	10	---	---	H
4	10	---	---	H
5	10	---	---	H
6	10	---	---	H
7	10	---	---	H
8	10	---	---	H
9	10	---	---	H
10	10	---	---	H
11	10	---	---	H
12	10	---	---	H

INVESTIGATOR: KulROOM NO. 105

DATE: _____

TECH: JT + CMSPECIES: Rabbit

ANIMAL I.D. #	WEIGHT (kg) ^g	OTHER
<u>1</u>	<u>1900</u>	
<u>2</u>	<u>1901.1</u>	
<u>3</u>	<u>2070.1</u>	
<u>4</u>	<u>2051.7</u>	
<u>5</u>	<u>2154.3</u>	
<u>6</u>	<u>2145.6</u>	
<u>7</u>	<u>2040</u>	
<u>8</u>	<u>2116</u>	
<u>9</u>	<u>2002.8</u>	
<u>10</u>	<u>1942</u>	
<u>11</u>	<u>1965.4</u>	
<u>12</u>	<u>2084</u>	

Rabbit's Initial Weights

in Kul

Date

Read and Understood By

J. Ham

Date

2nd Immunization -

7n weighed out the following: (whole resin)

RABCTP-2	3.5 mg	(2 ^{into} rabbit)
RABCTP-3	4.3	"
" - 4	4.7	"
" - 5	4.0	"
" - 6	3.8	"
" - 7	4.2	"

dissolved in 1.5 ml sterile PBS, pH 7.4.

According to Butz, S. et al. (1994) Peptide Research 7: 20-23
sonication of beads leads to partial breakage of
bead to make it more accessible to immune
system.

Solutions of beads were sonicated with nuclohp at
maximum setting for 2-3 minutes (minimum). Not
much change in turbidity of solution - beads still
intact & settle out quickly. Continued to
vibrate - each up to 5 minutes with little
change. (It is not clear how long the Butz paper
thons sonicated their beads).

left beads in PBS @ 4°C overnight.

7n warmed bead solution to room temperature.
added 1.5 ml INCOMPLETE Freund's Adjuvant (Sigma).
emulsified using 2 x 5cc syringes as before (p. 165).

immunized rabbit subcutaneously (~1.5 ml of each
solution) in multiple sites on back.

beads to be taken from

Signature

Aine Kruel

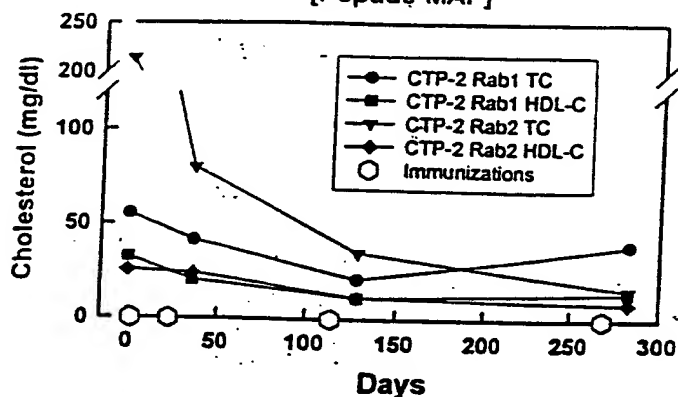
Date

Read and Understood By

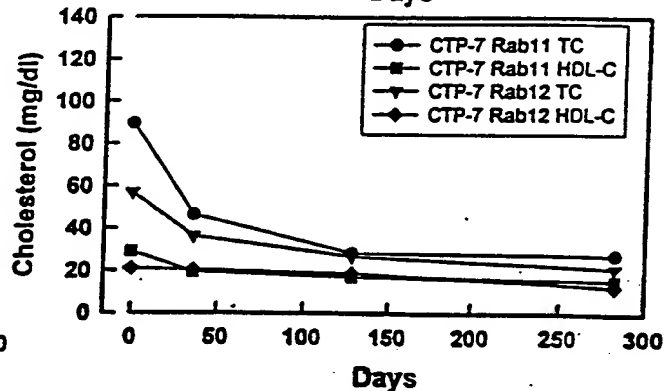
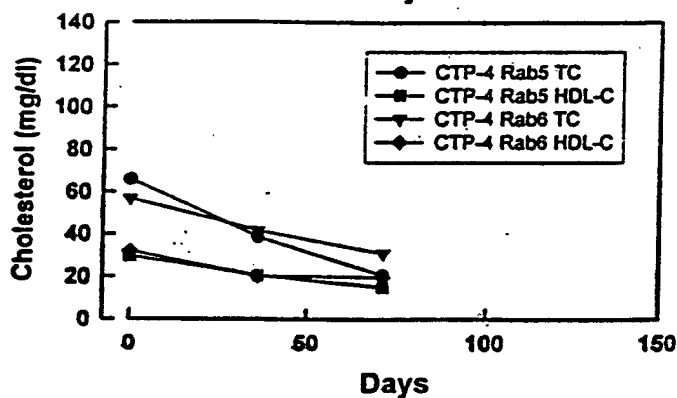
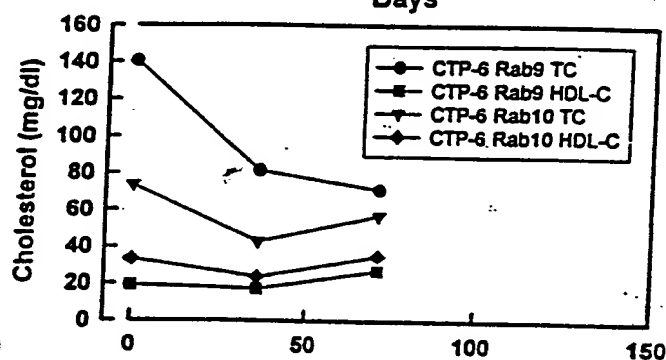
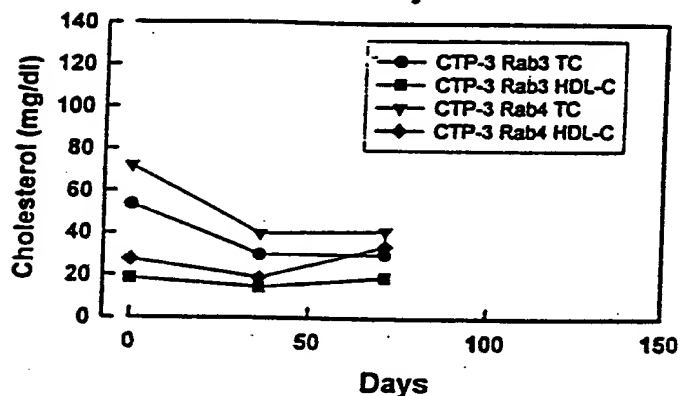
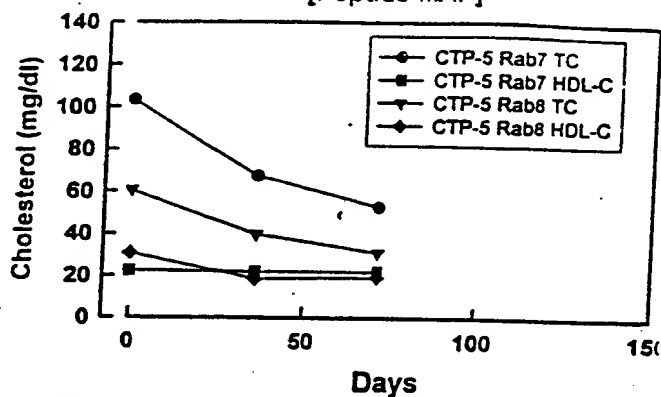
S. Kan

Date

Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]

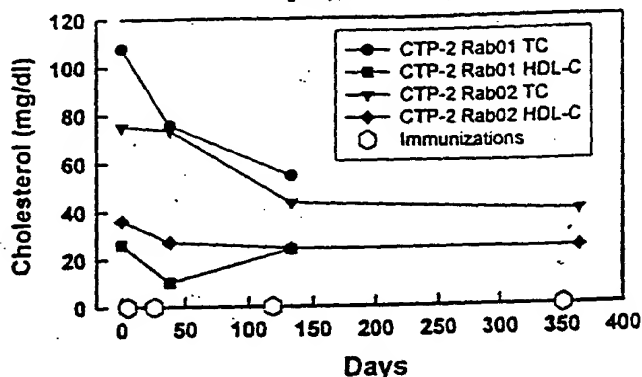


Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]

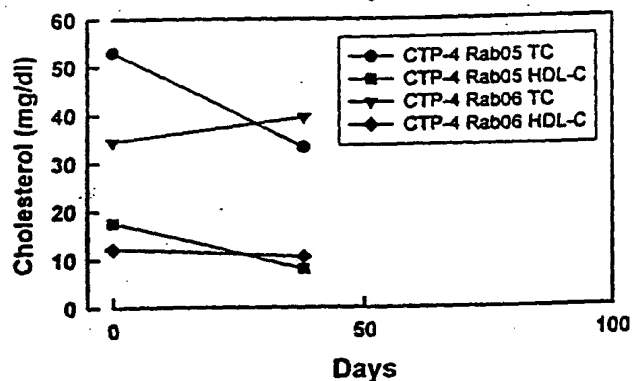
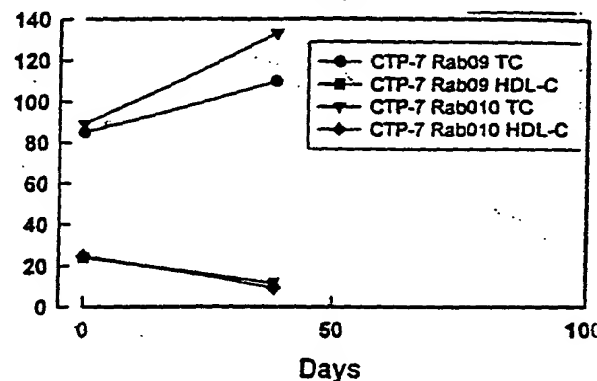
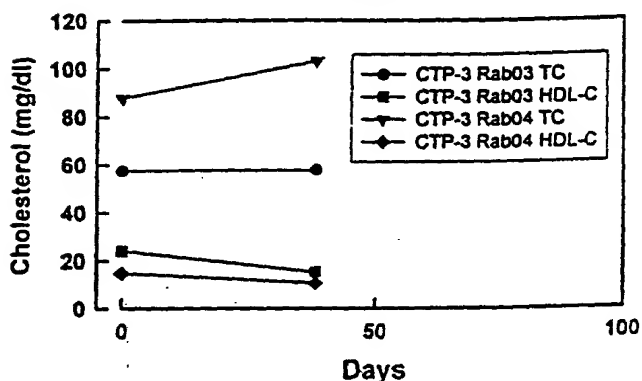
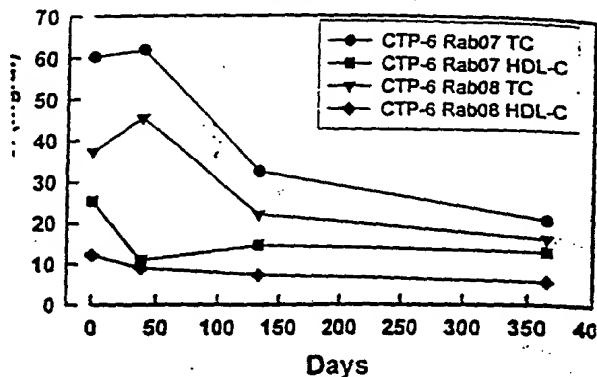


Conclusions: Initial drop in cholesterol may be adaptation to new diet (Vendor vs. Searle) or consequence of immunizing per se. Possible effect of CTP-3, CTP-6 immunizations in raising HDL. Curiously no Ab bound to peptide on ELISAs (see 5634011) for

Total & HDL Cholesterol in Immunized Rabbits [Peptide-PPD]



Total & HDL Cholesterol in Immunized Rabbits [Peptide-PPD]



*ELISA results
See

Conclusion: Drop in cholesterol seen over time. Not clear why. Only CTP-2 & CTP-6 showed Abs to peptide on ELISA*. One rabbit that died (CTP-2) appeared to have HDL raising. Also rabbit 07 (CTP-6) appeared to have HDL elevation. No effect on HDL seen w/ CTP-3, CTP-4 or CTP-7.

Author's Signature
Elaine Kuhl

Date

Read and Understood By

Denise Nachowiak

Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Needleman et al.)	Attorney Docket
)	6221/76429
Serial No.: 09/386,591)	Mon-103.0 CON I
)	
Filed: 31 Aug, 1999)	Art Group: 1642
)	
For: AN IMMUNOLOGICAL PROCESS AND)	
CONSTRUCTS FOR INCREASING)	
HDL CHOLESTEROL)	
CONCENTRATION BY DNA)	
VACCINATION)	

Examiner: J. Anders

November 22, 2000

EXHIBIT B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Needleman et al.)
Serial No.: 08/785,997) Attorney Docket
Filed: January 21, 1997) 6221/68346
For: AN IMMUNOLOGICAL PROCESS) MON-101.0
FOR INCREASING THE HDL) Art Group:
CHOLESTEROL CONCENTRATION) 1642
Examiner: T. Scheiner)

DECLARATION OF ELAINE KRUL
PURSUANT TO 37 C.F.R. §1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Dr. ELAINE KRUL Declares:

1. That she is presently employed by the Nutrition and Consumer Sector, a division of the Monsanto Company, and that she was formerly employed by G.D. Searle, Co., (Searle) a wholly owned subsidiary of the Monsanto Company, the assignee of the above-identified patent application;

2. That she received her Ph.D. degree from McGill University, Montreal, Quebec, Canada in 1982, majoring in biochemistry;

3. That she was employed by Searle between February of 1994 and February of 1998 doing laboratory research related to atherosclerosis;

4. That the work described in this Declaration was carried out in the State of Missouri, the United States of America, by one or more persons under her direction and control, or by her under the direction of one or both of the named inventors;

5. That the work described in this Declaration was completed on a date prior to November 7, 1996, the date of publication of WO 996/34888;

6. That the documents attached to this Declaration are true copies of one or more of her laboratory notebooks and an invoice, except that the dates and notebook numbers have been obscured for the purposes of this Declaration;

7. That collective Exhibit 1 contains pages 158, 159 and 160 from one of her notebooks (notebook A) that contains the amino acid residue sequence of cholesteryl ester transfer protein (CETP), using single letter code, in which peptide sequences corresponding to RABCTP-2, RABCTP-3, RABCTP-4, RABCTP-5, RABCTP-6, and RABCTP-7 are underlined and identified as such;

8. That those peptides of Paragraph 7 correspond to SEQ ID Nos: 2, 3, 4, 5, 6, and 7, respectively of the above-identified application;

9. That enclosed Exhibit 2 is a copy of page 161 of that same notebook that reiterates the names of those polypeptides and provides a schematic of a branched oligolysine antigenic carrier molecule referred to a Multiple Antigenic Peptide Backbone that when conjugated to a polypeptide is also referred to as MAP in her notebooks, and is noted at page 18, lines 11-19 of the above-identified application;

10. That enclosed Exhibit 3 is a copy of an invoice from Genosys Biotechnologies, Inc., of The Woodlands, Texas (Genosys) directed to Dr. Krul for conjugates of MAP-covalently-bonded polypeptides RABCTP-2, -3, -4, -5, -6 and -7;

11. That twelve rabbits were immunized on two occasions, two each, with a MAP-linked polypeptide conjugate received from Genosys;

12. That those two immunizations were recorded on pages 165, 166 and 167 of her above notebook, copies of which are enclosed as collective Exhibit 4;

13. That the samples of the immunized rabbits' blood were taken thereafter and were assayed for total cholesterol (TC) and high density lipoprotein (HDL-C);

14. That enclosed Exhibit 5 is a copy of page 071 of a second of her notebooks (notebook B) that contains graphs of the total cholesterol (TC) and high density lipoprotein (HDL-C) for each of the rabbits used in the study from prior to the immunizations (day zero) through at least 75 days thereafter, and in which the boxes within each graph identify each peptide with a shortened designation (CTP-number rather than RABCTP-number) because of space considerations;

15. That those data indicate a general lowering of both total cholesterol and high density lipoprotein for each of the peptides used;

16. That total cholesterol was determined in a standard laboratory assay using a 96-well microtiter plate kit commercially available from Wako Pure Chemical Industries, Inc.;

17. That high density lipoprotein was determined in a standard laboratory assay using a commercially available kit from Sigma Chemicals, Inc., of St. Louis, MO;

18. That enclosed Exhibit 6 is a copy of page 072 of her notebook B showing data for rabbits immunized with

conjugates of free polypeptides RABCTP-2, -3, -4, -6 and -7 (again referred to as CTP-2, CTP-3 etc.), also obtained from Genosys, that were individually covalently bonded to tuberculin purified protein derivative (PPD) to form conjugates, and prepared as discussed in Example 1B of the above-identified application, beginning at page 37;

19. That the results shown in Exhibit 6 were obtained following two immunizations with the conjugates using the assay procedures discussed in relation to Exhibit 5;

20. That all statements made herein of her own knowledge are true and all statements made on information and belief are believed to be true; and further, these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Enclosures

Exhibits 1-6

Nov. 24, 1998
Date

Elaine Krul
Elaine Krul

CERTIFICATE OF MAILING

I hereby certify that this Declaration, its enumerated Exhibits, a related response, a Petition under 37 C.F.R. §1.136 and its fee, and a Petition under 37 C.F.R. §1.48 and its fee are being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on November 25, 1998.

Edward P. Gamson
Edward P. Gamson

RABBIT CETP SEQUENCE - RABCTP-2

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQAGYPDVSGERAVM
 10 20 30 40 50
 LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVFKGTLNYS
 60 70 80 90 100
 YTSAWGLGINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL
 110 120 130 140 150
 LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADVFQT
 160 170 180 190 200
 RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF
 210 220 230 240 250
 PPGLLGDSRMLYFWFSDQVLNSLARAFAEQEGRVLVSLTGDEFKKVLETQG
 260 270 280 290 300
 FDTNQEIFQELSRGLPTGQAQVAVHCLKVPKISCONRGVVVSSSVAVTFR
 310 320 330 340 350
 FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCVFASGRAGSS
 360 370 380 390 400
 ANLSVALRTEAKAVSNLTESRSESLQSSLRSLIATVGIPEVMSRLEVAFT
 410 420 430 440 450
 ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHLLVDLQSL
 460 470 480 490

SEARLE

Rabbit Anth-CETP Project
Choice of Peptides for Immunization

159

RABBIT CETP SEQUENCE - RABCTP-3 / RABCTP-4

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQRAGYPDVSGERAVM

10	20	30	40	50

LLGRVKYGLHNLQISHLSIASSQVEIVDAKTIDVAIQNVSVVFKGTLNYS

60	70	80	90	100

YTSAWGLGINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL

110	120	130	140	150

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFVQT

160	170	180	190	200

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF

210	220	230	240	250

PPGLLGDSRMLYFWFSDQVLNSLARAAFQEGRLVLSLTGDEFKKVLETQG

260	270	280	290	300

RABCTP-3

FDTNQEIFQELSRGLPTGQAQVAVHCLKVPKISCQNRGVVVSSSVAVTER

310	320	330	340	350

FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCVPASGRAGSS

360	370	380	390	400

ANLSVALRTEAKAVSNLTESRSESLQSSLRSLIATVGIPEVMSRLEVAFT

410	420	430	440	450

ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHLLVDFLQSL

460	470	480	490

RABCTP-4

Rabbit anti-CETP Project
Choice of Peptides for Immunization

SEARLE

RABBIT CETP SEQUENCE - RABCTP-5 / RABCTP-6 / RABCTP-7

RABCTP-7

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQ^{RAGY}PDVSGERAVM

10 20 30 40 50

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVFKGTLNYS

60 70 80 90 100

YTSAWGLQINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL

110 120 130 140 150

RABCTP-6

LLHLOGEREPGWLKQLETFNFI^{SFTL}KLILKGQVCNEINTISNIMAD^{FVQT}

160 170 180 190 200

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF

210 220 230 240 250

PPGLLGDSRMLYFWFSDQVLNSLARA^{AFQ}EGRVLVSLTGDEFKKVLETQG

260 270 280 290 300

FDTNQEIFQELSRGLPTGQAQVAVHCLKV^{PKIS}CQNRGVVSSSVAVTFR

310 320 330 340 350

RABCTP-5

FPRPDGREAVAYRFEEDII^{TTVQ}ASYSQKKLFLHLLD^{FQ}CVPASGRAGSS

360 370 380 390 400

ANLSVALRTEAKAVSNLTESRSESLOSSLRSLIATVGIPEVMSRLEVAFT

410 420 430 440 450

ALMNSKGLDLFEIINPEIITLDGCLLLQMDFGFPKHL^{LVDF}LQSL

460 470 480 490

SEARLE

Rabbit anti-CETP Project
Choice of Peptides for Immunization

161

Summary of Peptide Sequences Submitted to Genosys
to couple on MAP:

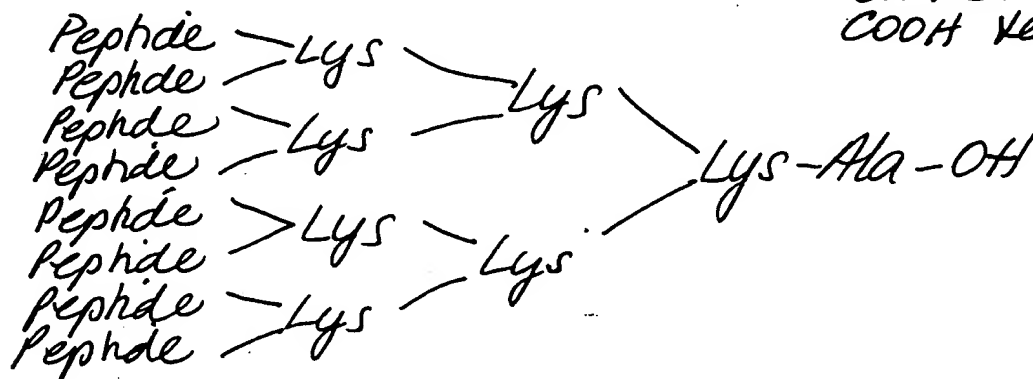
RABCTP-2	306 - 325	(#001)	Order # 054215 P.O.# 8002455
RABCTP-3	345 - 364	(#002)	40µm of each made - Immuno
RABCTP-4	475 - 496	(#003)	Grade (>60% pure)
RABCTP-5	370 - 389	(#004)	Made on MAPS Resin: See reference: Butz, S. et al. (1994) Peptide Research 7: 20-23.
RABCTP-6	150 - 169	(#005)	
RABCTP-7	42 - 61	(#006)	

Decided to try coupling to Multiple Antigenic Peptide Backbone as a way to avoid "carrier" protein which will generate a lot of antibodies of and in itself.
One of the original references to this method is:

Posnett, DN, ~~McGrath~~ McGrath H, & Tam JP (1988)
J. Biol. Chem. 263: 1719-1725.

Structure of MAP:

NOTE: Peptides are
linked via their
COOH termini!!



GENOSYS

Genosys Biotechnologies, Inc.
1442 Lake Front Circle, Suite 185
The Woodlands, TX 77380-3600
(713) 363-3693
FAX (713) 363-2212

Order No.

Page 2

ORDER

54215

Bill To
MONSANTO COMPANY
ACCOUNTS PAYABLE, N2F
800 NORTH LINDBERGH BLVD.

ST. LOUIS MO 63167

Ship To
DR. ELAINE KRULL
MONSANTO COMPANY
60-000-760.92
T213W/T2M
800 N. LINDBERGH BLVD.
ST. LOUIS MO 63167

---CONTACT---
BARB GRIFFARD
314-694-1000 X 6825

---CONTACT---
314-694-4218

Customer: 100 6316701 KRU1
Salesman: 8
Terms: NET 30
SHIP VIA: Airborne (Prepaid)
O Number: B002455

ORDERED:

SHIPPED:
INVOICED:

QTY	ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC	NET PRICE	EXTENSION
5.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 uM IMMUNO GRADE RABCTP-6 LLL HLG GER EPK MLK QLF TH RESIDUES IMMUNO GRADE MAPS RESIN	20.00	35.00		950.00	
MW 2393 + P157A-030 phide 54215-005									
6.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 uM IMMUNO GRADE RABCTP-7 DVS GER AVH LLG RVK YGL HH RESIDUES IMMUNO GRADE MAPS RESIN	20.00	35.00		950.00	
MW 2215 ot P157A-032 ephide 54215-006									

COMMENTS:
cleaned by 85% TPA, ether extracted 3X, suspended in
10% TPA/H₂O - 1-2% acetic acid resuspend
+ lyophilized
lyophilized

SALES TAX
MISCELLANEOUS
FREIGHT
TAX
4.624.00

TOTAL

PACKING LIST

GENOSYS

Genosys Biotechnologies, Inc.
1442 Lake Front Circle, Suite 185
The Woodlands, TX 77380-3600
(713) 363-3693
FAX (713) 363-2212

1-800-234-5362
Stacey ext #127
Hogue

[615-343-1465]
James Tam

Order No.

37mm/g
0.37µm/mg norm

Dr. Stanfield

Page 1
713-363-3693 ext 13

ORDER

54215

Bill To
MONSANTO COMPANY
ACCOUNTS PAYABLE, N2F
800 NORTH LINDBERGH BLVD.

Ship To
DR. ELAINE KRULL
MONSANTO COMPANY
60-000-760.92
T213W/T2M
800 N. LINDBERGH BLVD
ST. LOUIS MO 63167

ST. LOUIS

MO 63167

CONTACT
BARB GRIFFARD
314-694-1000 X 6825

CONTACT
314-694-4218

Customer: 100 6316701 KRU1
Salesman: 8
Terms: NET 30
SHIP VIA: Airborne (Prepaid)
O Number: 8002455

ORDERED:

SHIPPED:

INVOICED:

ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC	NET PRICE	EXTENSION
1.000	1.00	0.00 PEP1G/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-2 EIF QEL SRG LPT GQA QVA VH RESIDUES IMMUNO GRADE MAPS RESIN MW 2181 ot P157A-022 hde # 54215-001 ~87 mg	20.00	35.00		950.00	
1.000	1.00	0.00 PEP1G/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-3 VAV TFR FPR PDG REA VAY RF RESIDUES IMMUNO GRADE MAPS RESIN MW 2355 ot P157A-024 ephde # 54215-002 ~94 mg	20.00	35.00		950.00	
1.000	1.00	0.00 PEP1G/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-4 LLL QMD FGF PKH LLV DFL QSL S RESIDUES IMMUNO GRADE MAPS RESIN PEP SYN MW 2562 ot # P157A-026 ephde # 54215-003 ~102 mg	22.00	55.00		1,030.00	
1.000	1.00	0.00 PEP1G/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-5 TTV QAS YSQ KXL FLH LLD FQ RESIDUES IMMUNO GRADE MAPS RESIN MW 2369 Lot P157A-028 ephde # 54215-004 ~95 mg	20.00	35.00		950.00	

ACKING LIST

CONTINUE

Immunization of Rabbits

Subcutaneously administered (0.05-0.1 ml/site)
along rabbit's back - done by Margi Baldwin.

Rabbits in Room 105 (Tattooed)

#1, #2	RABCTP-2	(3.3 mg total ÷ 2 rabbits)	
#3, #4	RABCTP-3	(3.4 mg ")
#5, #6	RABCTP-4	(3.2 mg ")
#7, #8	RABCTP-5	(3.2 mg ")
#9, #10	RABCTP-6	(3.3 mg ")
#11, #12	RABCTP-7	(3.5 mg ")

INVESTIGATOR: _____ DATE: _____
 room #: _____ phone # _____ Mail Zone: 2am
 Technician: _____

5-
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a
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d
en in
uob
20°C.

Animal ID #	Bleed	Serum/Plasma	Other	Initials
1	10	---	---	HH
2	10	---	---	QH
3	10	---	---	HH
4	10	---	---	HH
5	10	---	---	QH
6	10	---	---	HH
7	10	---	---	QH
8	10	---	---	HH
9	10	---	---	QH
10	10	---	---	HH
11	10	---	---	QH
12	10	---	---	HH

INVESTIGATOR: KaulROOM NO. 105

DATE: _____

TECH: JT+GMSPECIES: Rabbit

ANIMAL I.D. #	WEIGHT (lbs/kg) g	OTHER
<u>1</u>	<u>1900</u>	<u> </u>
<u>2</u>	<u>1901.1</u>	<u> </u>
<u>3</u>	<u>2070.1</u>	<u> </u>
<u>4</u>	<u>2051.7</u>	<u> </u>
<u>5</u>	<u>2154.3</u>	<u> </u>
<u>6</u>	<u>2145.6</u>	<u> </u>
<u>7</u>	<u>2040</u>	<u> </u>
<u>8</u>	<u>2116</u>	<u> </u>
<u>9</u>	<u>2002.8</u>	<u> </u>
<u>10</u>	<u>1942</u>	<u> </u>
<u>11</u>	<u>1965.4</u>	<u> </u>
<u>12</u>	<u>2084</u>	<u> </u>

Rabbit's Initial Weights

ure

ine Kaul

Date

Read and Understood By

J. Hays

Date

EARLE

cond Immunization -

On weighed out the following: (whole resin)

RABCTP-2	3.5 mg	(2 ^{into} rabbits)
RABCTP-3	4.3	"
" - 4	4.7	"
" - 5	4.0	"
" - 6	3.8	"
" - 7	4.2	"

Dissolved in 1.5 ml sterile PBS, pH 7.4.

According to Butz, S. et al. (1994) Peptide Research 7: 20-23 sonication of beads leads to partial breakage of bead to make it more accessible to immune system.

Solutions of beads were sonicated with microtip at maximum setting for 2-3 minutes (minimum). Not much change in turbidity of solution - beads still look intact & settle out quickly. Continued to sonicate - each up to 5 minutes with little change. (It is not clear how long the Butz paper authors sonicated their beads).

Left beads in PBS @ 4°C overnight.

In warmed bead solution to room temperature. Added 1.5 ml INCOMPLETE Freund's Adjuvant (Sigma). Emulsified using 2 x 5cc syringes as before (p. 165).

Immunized rabbits subcutaneously (~1.5 ml of each emulsion) in multiple sites on back.

Bloods to be taken from

Signature

Saini Kaul

Date

Read and Understood By

S. Kaul

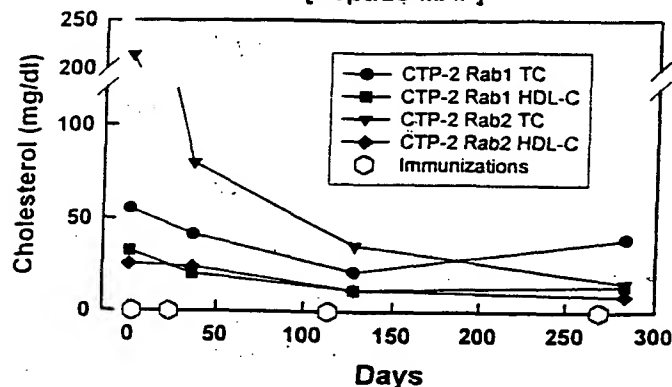
Date

SEARLE

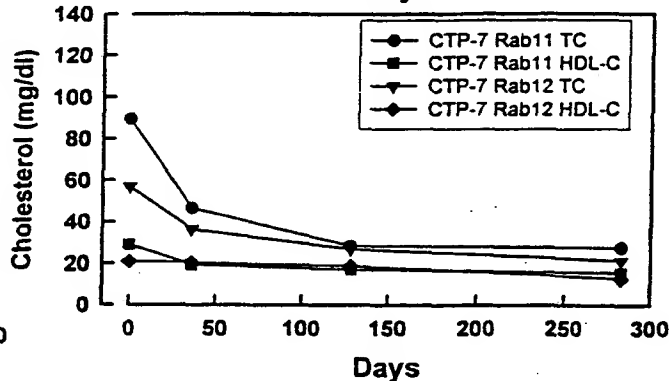
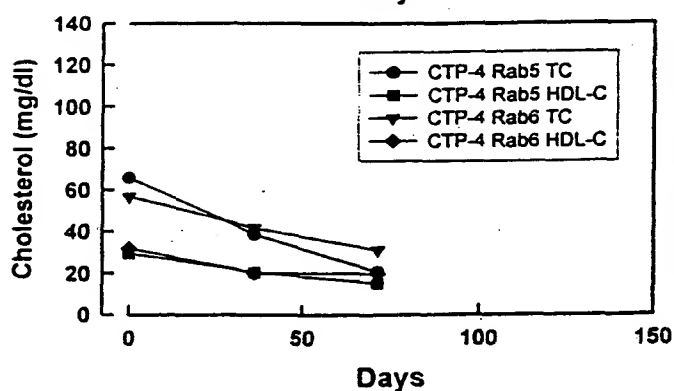
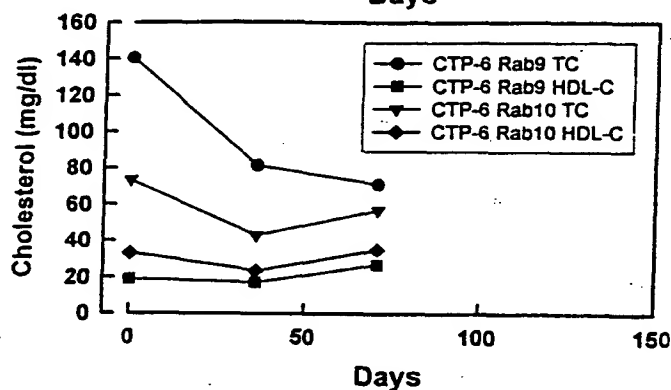
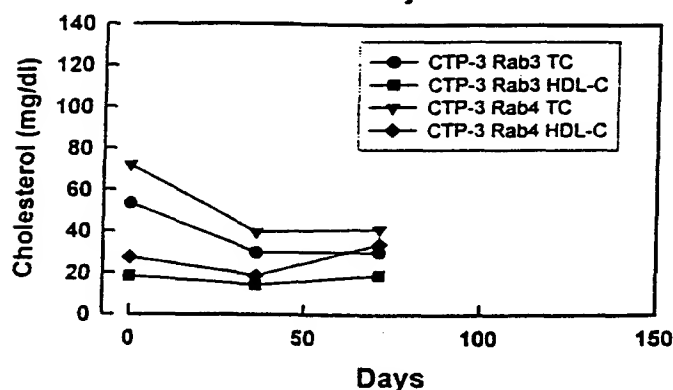
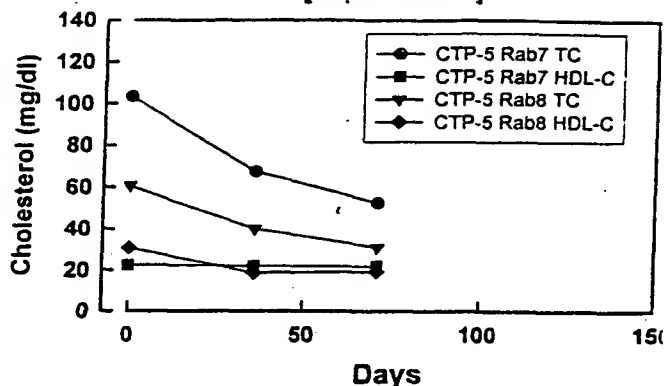
Summary of Lipid Report on
Antisera from CTP-Peptide
Immunized Rabbits

071

Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]



Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]



Conclusions: Initial drop in cholesterol may be adaptation to new diet (Vendor vs. Searle) or consequence of immunizing per se. Possible effect of CTP-3, CTP-6 immunizations in raising HDL. Curiously no Ab bound to peptide on ELISAs (see 5634011) for

Author's Signature

Elaine Kuhl

Date

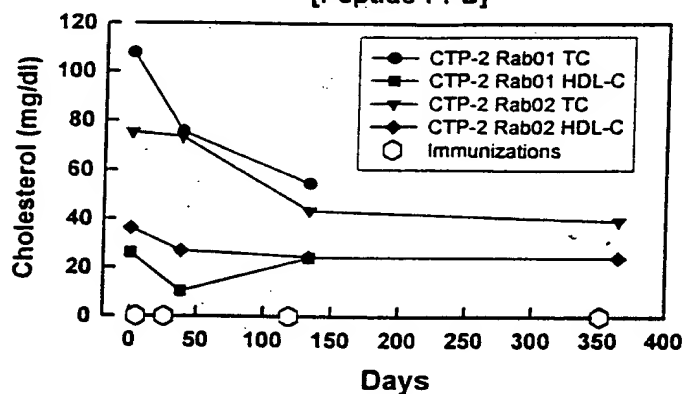
Read and Understood By

Denise Nachowiak

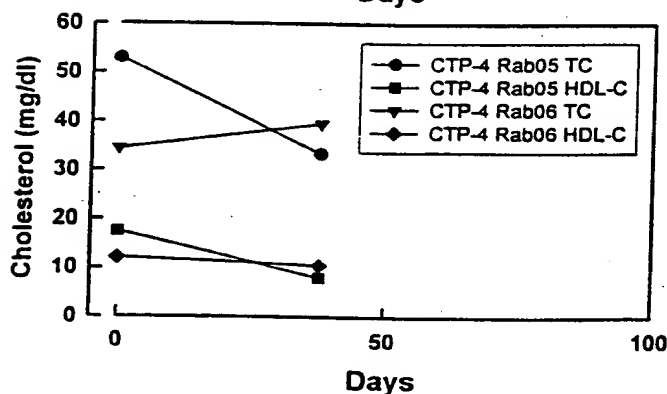
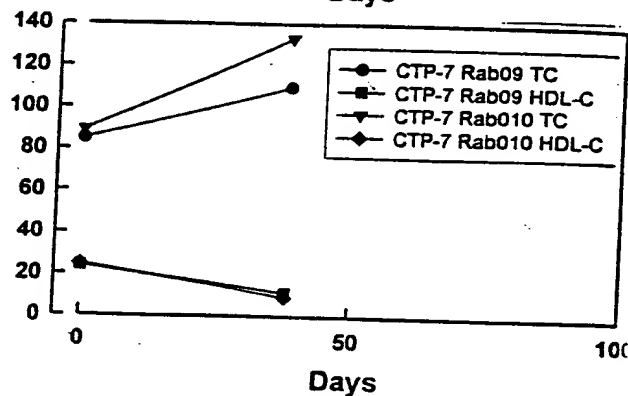
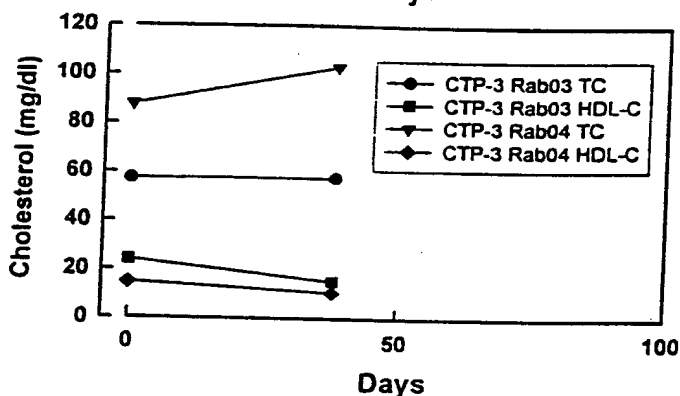
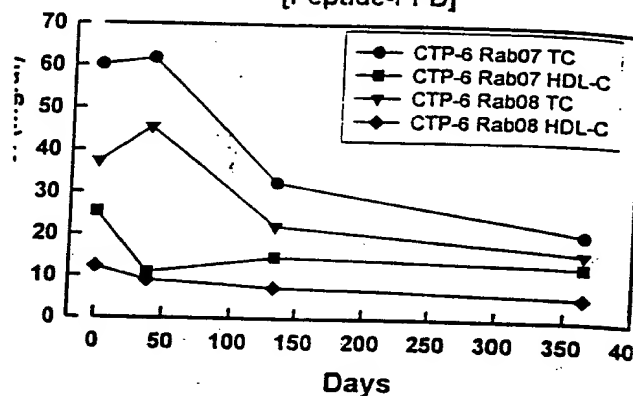
D

Book Number	Subject	Project Number
Page	Summary of Lipid Assays on Antisera from CTP-Peptide Immunized Rabbits	SEARLE
072		

Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-PPD]



Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-PPD]



*ELISA results
See

Conclusion: Drop in cholesterol seen over time. Not clear why. Only CTP-2 & CTP-6 showed Abs to peptide on ELISA* One rabbit that died (CTP-2) appeared to have HDL raising. Also rabbit 07 (CTP-6) appeared to have HDL elevation. No effect on HDL seen in CTP-3, CTP-4 or CTP-7.

Author's Signature	Date	Read and Understood By	Date
Elaine Kuhl		Denise Nachowiak	